Genetic variation in fall cold hardiness in coastal Douglas-fir in western Oregon and Washington

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Abstract: Genetic variation in fall cold damage in coastal Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco var. *menziesii*) was measured by exposing excised branches of seedlings from 666 source locations grown in a common garden to freezing temperatures in a programmable freezer. Considerable variation was found among populations in fall cold hardiness of stems, needles, and buds compared with bud burst, bud set, and biomass growth after 2 years. Variation in fall cold hardiness was strongly correlated ($r = 0.67$) with cold-season temperatures of the source environment. Large population differences corresponding with environmental gradients are evidence that natural selection has been important in determining genetic variation in fall cold hardiness, much more so than in traits of bud burst (a surrogate for spring cold hardiness), bud set, and growth. Seed movement guidelines and breeding zones may be more restrictive when considering genetic variation in fall cold hardiness compared with growth, phenology, or spring cold hardiness. A regional stratification system based on ecoregions with latitudinal and elevational divisions, and roughly corresponding with breeding zones used in Oregon and Washington, appeared to be adequate for minimizing population differences within regions for growth and phenology, but perhaps not fall cold hardiness. Although cold hardiness varied among populations, within-population and within-region variation is sufficiently large that responses to natural or artificial selection may be readily achieved.

Key words: cold hardiness, genetic variation, adaptation, *Psuedotsuga menziesii*.

Résumé : Les auteurs ont mesuré la variation génétique des dommages par le froid automnal, chez le sapin Douglas (*Pseudotsuga menziesii* (Mirb.) Franco var. *menziesii*), en exposant les rameaux excisés de plantules provenant de 666 localités sources, cultivées dans un jardin commun, à des températures de congélation dans un congélateur programmable. On a trouvé une variation considérable au sein de la population quant à la résistance au froid automnal chez les tiges, les aiguilles et les bourgeons, comparativement à l'ouverture des bourgeons et la croissance de la biomasse après deux ans. La variation de la résistance au froid automnal est fortement corrélée ($r = 0{,}67$) avec les températures de la saison froide de l'environnement source. Les grandes différences observées dans les populations correspondant aux gradients environnementaux, sont des preuves que la sélection naturelle a joué un rôle important dans la détermination de la variation génétique de la résistance au froid automnal, beaucoup plus que pour les caractères de l'ouverture des bourgeons (l'équivalant de la résistance au froid printanier), la formation des bourgeons, et la croissance. Les prescriptions pour le mouvement des graines et les zones de croisement pourraient être plus restrictives, lorsqu'on considère la variation de la résistance au froid automnal comparativement à la croissance, la phénologie, ou la résistance au froid printanier. Un système de stratification régional, basé sur des écorégions avec des divisions latitudinales et altitudinales correspondant grossièrement aux zones de croisement utilisées en Oregon et Washington, semble adéquat pour minimiser les différences dans les régions, quant à la croissance et la phénologie mais possiblement pas pour la résistance au froid automnal. Bien que la résistance au froid varie entre les populations, dans les populations et dans les régions, la variation est suffisamment importante pour que les réactions à la sélection naturelle ou artificielle se réalisent rapidement.

Mots clés : résistance au froid, variation génétique, adaptation, *Pseudotsuga menziesii*.

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Introduction

Susceptibility to damage from cold is important to the adaptation of Douglas-fir (*Psuedotsuga menziesii* (Mirb.) Franco), particularly because of the highly variable environments within its range, both spatially and temporally. Cold hardiness varies among populations of Douglas-fir, and much of the variation is clinally related to gradients in temperature and moisture (Campbell and Sorensen 1973; Rehfeldt 1979, 1986; White 1987; Loopstra and Adams 1989). Large population differences and a consistent, strong association of a trait with environments provide indirect evidence that a trait may be adaptive and that natural selection was important in shaping variation (Endler 1986). Cold hardiness traits also vary considerably within populations, and this variation may be subject to natural or artificial selection (e.g., tree improvement programs). Bud burst and spring cold hardiness in coastal Douglas-fir (var. *menziesii*) are under strong genetic control and highly genetically correlated (Aitken and Adams 1997; O'Neill et al. 2000, 2001). Heritabilities for bud set and cold hardiness in the fall and winter are low to moderate (Aitken et al. 1996; Aitken and Adams 1997; O'Neill et al. 2000, 2001). Correlations between bud set and fall cold hardiness vary among studies,

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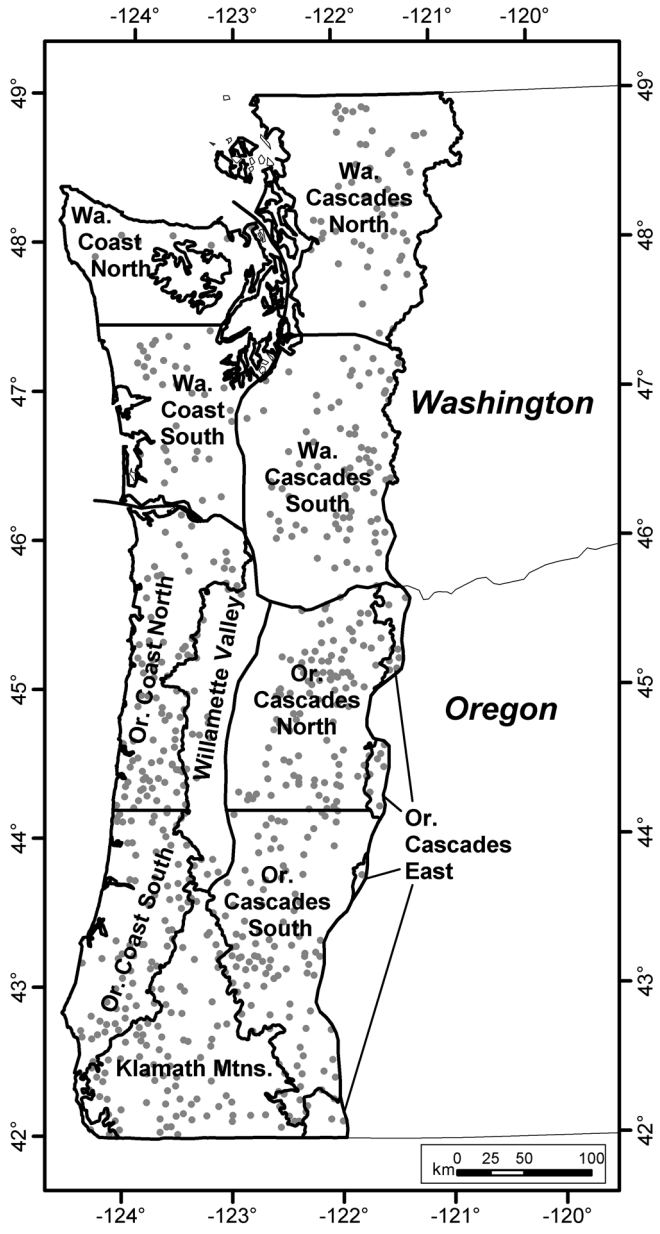
Fig. 1. Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*) source locations and regional classification used in analyses. Each region was further divided into high and low elevations at 650 m (not shown).



but are often weak (Aitken et al. 1996; O'Neill et al. 2000). Over a large geographic and climatic scale, tradeoffs exist between cold hardiness and growth, although at smaller scales, the correlations are weaker and less consistent (Howe et al. 2003); a strong correlation was found between fall cold injury and height ($r = 0.70$) in seedlings among interior Douglas-fir (var. *glauca*) populations in northern Idaho (Rehfeldt 1979), whereas most genetic and family mean correlations were near zero for fall cold injury and height in coastal Douglas-fir saplings in two Washington breeding zones (Aitken et al. 1996). Stevenson et al. (1999) found that genetically improved Douglas-fir seedlings were less cold hardy than unimproved seedlings grown at two sites in British Columbia.

Previous studies of genetic variation in cold hardiness in coastal Douglas-fir have included only a few populations or breeding zones, or have been of limited geographic range. Thus, conclusions about the structure and patterns of genetic variation are incomplete. In this study, I report on genetic variation in fall cold hardiness using artificial freeze testing on a large number of families distributed across much of the range of coastal Douglas-fir in western Oregon and Washington. The objective is to explore structure and patterns of genetic variation in fall cold hardiness by considering the relative magnitudes of among- and within-population variation and the relationship of population variation to environmental variation. In so doing, I hope to evaluate the importance of cold hardiness in determining adaptation of Douglas-fir populations to their local environments. I also considered the effect of geographic scale to conclusions about the magnitudes of variation and relationships to environment by dividing the study area into regions. This also allowed comparisons among regions in means, family variances, and correlations, and facilitated comparisons with other studies done at the scale of breeding zones.

Materials and methods

Common garden test

Samples for this study are part of a larger study of the genecology of coastal Douglas-fir that focuses on geographic variation in traits of emergence, bud phenology, growth, and partitioning as measured on seedlings grown in a common garden (St. Clair et al. 2005). The present study reports on genetic variation in fall cold hardiness as measured by artificial freeze tests on approximately two-thirds of the seedlings from the earlier study. To allow comparisons of fall cold hardiness to other traits, bud phenology and seedling biomass are reanalyzed using the same subset of seedlings. Spring cold damage was not measured, since only two branches per seedling could be destructively sampled, and because bud burst appears to be a surrogate for spring cold damage based on findings of high genetic correlations in previous studies (Aitken and Adams 1997; O'Neill et al. 2000). Bud set, however, does not appear to be strongly correlated to fall cold hardiness (Aitken et al. 1996; Aitken and Adams 1997; O'Neill et al. 2000, 2001).

The sampling design for parents from native stands and common garden procedures are described by St. Clair et al. (2005). In brief, wind-pollinated seeds were collected from parent trees in naturally regenerated stands throughout the range of Douglas-fir in western Oregon and Washington. Progeny from the parents were grown for 2 years in raised nursery beds in Corvallis, Oregon. To evaluate a large number of parent trees, tests were established in 3 successive years (1994–1996) using different sets of families, but with a common set of families included in all 3 years to allow for adjustment of year effects (see White and Hodges 1989). Each year families were randomly assigned to five-tree row plots (of which four trees were used for cold hardiness testing) in each of four raised beds with each bed treated as a block. A total of 792 families from 666 source locations (i.e., populations) were evaluated for cold hardiness over the 3 establishment years, with 10 families measured in all 3 years.

Table 1. Results from analyses of variance for differences among Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*) seed source locations and families-within-locations across western Oregon and Washington.

					Percentage of total variance			
Trait	Overall mean	*F* value for locations	*F* value for family-within-locations	Total variance	Location	Family (*L*)	Error	Q_{st}[a]
Stem cold damage (%)	24.9	4.79***	1.51***	380.7	51	4	45	0.66
Needle cold damage (%)	45.2	4.48***	1.92***	579.7	53	8	40	0.53
Bud cold damage (%)	48.5	4.52***	1.43**	404.5	49	4	47	0.67
Bud burst (d)[b]	107	1.73***	3.59***	30	27	25	48	0.15
Bud set – year 1 (d)[b]	274	2.72***	2.11***	99	37	13	51	0.33
Bud-set – year 2 (d)[b]	225	2.18***	1.58***	418	26	8	67	0.36
Biomass (g)	12.5	1.69***	1.92***	17.3	16	16	68	0.14

Note: ***, statistically significant at $p < 0.001$); **, statistically significant at $p = 0.01$–0.001.

[a]Defined as the proportion of the total genetic variation in quantitative traits found among populations (see text).

[b]Number of days since 1 January.

Cold hardiness testing

Branch samples were taken for cold hardiness testing in the fall after the second growing season, and subjected to artificial freeze tests following methods described by Anekonda et al. (2000). The method involved removing 4–6 cm long shoot tips from two lateral branches of each seedling, with each branch labeled to ensure identity. Branches from 50 seedlings were wrapped in a packet of moist cheesecloth and aluminum foil with each of the two branches of a seedling in a separate packet. Branches were frozen in a programmable freezer at two different test temperatures chosen to give between 30% and 70% damage (one temperature for each branch sample from a seedling). Packets were placed in the freezer at –2 °C for approximately 10 h to allow the samples to equilibrate and to freeze extracellular water. The temperature was then lowered 3 °C per h to the target test temperature and maintained at that temperature for 1 h. After treatment, packets were removed from the freezer and put in a 4 °C refrigerator overnight to allow them to slowly thaw. The packets were then placed at room temperature for 6–7 d to allow cold injury symptoms to develop and become visible. The two test temperatures were chosen based on a preliminary test of cold hardiness made on 24 random seedlings from a range of sources sampled the previous week using four test temperatures.

Damage from freezing was visually scored for each tissue type as the percentage of tissue showing injury. Needle damage was scored as the percentage of needles that were brown or had fallen off the stems. Stem damage was scored by exposing a section of tissue with a tangential cut and noting the percentage of cambium and phloem that had turned color from healthy (whitish-green) to damaged (yellow or brown). Bud damage was scored by cutting open the bud and noting the percentage of bud tissue that had turned color from healthy (green) to damaged (yellow or brown). Damage was scored to the nearest 10%.

The large geographic scale of this study required many samples for fall cold hardiness testing. To get around practical limitations of freezer size and time required for scoring damage, samples were taken at four different dates spaced 2 weeks apart during October and November. A single seedling per family row-plot from each of four replications was sampled at each time; thus, four seedlings of a family row-plot per replication were sampled, giving a total of 16 seedlings per family. Test temperatures ranged from –11 °C and –13 °C for samples taken in early October to –26 °C and –29 °C for samples taken in late November. The damage score for an individual seedling was the average of the two branches frozen at the two different test temperatures. Analyses were done on a plot means basis, with scores for a plot being the average over the four sample dates. Damage scores of plot means were normally distributed, and no transformations were used. Previous studies have shown that genetic correlations of cold damage among fall sampling dates are high (Aitken and Adams 1996; O'Neill et al. 2001).

Analysis

Differentiation in cold hardiness traits was evaluated by partitioning family variance among and within populations using variance components estimated from the restricted maximum likelihood (REML) method. In this study, within-population variation was defined as variation among families within a source location, which was estimated by paired family samples at approximately one-fifth of the locations. The two families at a source location were taken from a similar elevation and aspect, but were separated by approximately 400 m to minimize relatedness among parents. Year effects were removed by standardizing plot means such that means and standard deviations for the check-lot families were equal across years (White and Hodges 1989). The model for the analysis was:

$$[1] \qquad Y_{ijk} = \mu + B_k + L_j + F(L)_{ij} + e_{ijk}$$

where Y_{ijk} is the plot mean performance of the ith family (F) from the jth source location (L) in the kth replication (B), μ is the overall experimental mean, and e is the experimental error consisting of the pooled interactions of both sources and families by replications. Source locations and families were treated as random effects. Differences among locations and families within locations were tested for significance using PROC GLM of the SAS statistical package (SAS Institute Inc. 1999). Location differences were tested using families within locations as the error term, and family differences were tested against the experimental error term. Variance components were obtained using PROC MIXED.

Table 2. Means overall and within each region for traits measured on Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*).

Region and elevation	No. of families	Stem damage (%)[a]	Needle damage (%)[a]	Bud damage (%)[a]	Bud burst (d)[b]	Bud set (d)[b]	Biomass (g)
All of western Oregon and Washington	792	24.9	45.2	48.5	106	274	12.5
Within regions							
Oregon Klamath Mountains low	47	41.0	70.0	63.4	102	278	13.9
Oregon Klamath Mountains high	56	35.3	64.3	57.1	104	273	12.5
Oregon Coast south low	74	48.9	71.5	75.1	105	282	13.0
Oregon Coast south high	13	47.3	67.9	73.2	104	280	14.0
Oregon Coast north low	86	35.5	55.3	59.2	108	278	13.0
Oregon Coast north high	14	32.3	45.9	57.6	108	278	11.4
Oregon Coast eastside low (Willamette Valley)	27	31.2	54.0	57.3	103	279	14.0
Washington Coast south low	41	18.5	34.9	42.8	113	276	14.3
Washington Coast north low (Olympics)	14	13.8	31.0	38.4	108	273	11.5
Oregon Cascades south low	26	23.4	48.7	44.0	106	276	13.2
Oregon Cascades south high	83	18.6	40.3	40.5	106	268	11.1
Oregon Cascades north low	55	19.2	40.4	42.8	107	277	12.8
Oregon Cascades north high	73	15.4	32.9	39.9	108	268	9.9
Oregon Cascades eastside high	26	10.6	24.0	35.5	106	261	10.1
Washington Cascades south low	35	17.9	37.0	40.4	110	276	13.5
Washington Cascades south high	49	12.2	28.2	36.6	108	269	12.0
Washington Cascades north low	44	12.7	27.7	36.7	108	275	14.0
Washington Cascades north high	29	12.8	30.2	37.1	107	271	12.7

[a]Percentage of tissue damaged.

[b]Number of days since January 1st.

Q_{st} was used as a measure of population differentiation. Q_{st} is the proportion of the total genetic variation for quantitative traits that is found among populations, and is estimated as (Prout and Barker 1993; Spitze 1993):

$$[2] \qquad Q_{st} = \sigma^2_p/[\sigma^2_p + 2\sigma^2_{w(p)}]$$

where σ^2_p is the additive genetic variance among populations as estimated by the variation among source locations, and $\sigma^2_{w(p)}$ is the additive genetic variance within populations as estimated by $3 * \sigma^2_{f(p)}$, where $\sigma^2_{f(p)}$ is the variance component for open-pollinated families within locations. A coefficient of 3 was chosen because genetic relatedness of open-pollinated families is expected to be somewhat greater than half-sibs (Campbell 1979).

I did a second type of analysis where I assigned all families to regions using a stratification system that included ecoregions, latitudal divisions within ecoregions, and elevational divisions within the ecoregion and latitudal strata (Fig. 1). The purpose of this stratification system was to explore geographic differences in means, family variances, and correlations among traits. The stratification system was chosen to reflect known general patterns of variation (St. Clair et al. 2005). Furthermore, the stratification approximates breeding zones used in breeding programs in Oregon and Washington, and allows comparisons to earlier studies using materials from individual breeding programs. Ecoregions denote areas of similar ecosystems including similarity in geology, physiography, vegetation, climate, soils, land use, wildlife distributions, and hydrology. I used Omernik's level III ecoregions (Omernik 1995; Pater et al. 1998). These ecoregions included the Klamath Mountains, Coast Range, Willamette Valley, Cascades, Eastern Cascades Slopes and Foothills, and North Cascades. The Coast Range ecoregion was further divided at 44.2°N latitude, at the Columbia River (about 46.2°N latitude), and at 47.8°N latitude. The Cascade ecoregion was further divided at 44.2°N latitude and at the Columbia River (about 45.6°N latitude). All ecoregion-latitudinal strata were further divided into low and high elevations at 650 m, although the Washington Coast Range and the Willamette Valley strata did not have high-elevation sources, and the Eastern Cascades strata did not have low-elevation sources. In some cases (in the Puget Sound, eastern Willamette Valley, and southeastern Washington Cascades) the few families in an ecoregion were assigned to adjacent ecoregions.

The analysis of variance including regions used the model:

$$[3] \qquad Y_{ijkl} = \mu + B_i + R_j + L(R)_{jk} + F(L)_{kl} + e_{ijkl}$$

where Y_{ijkl} is the plot mean performance of the lth family (F) from the kth source location (L) from the jth region (R) in the ith replication (B), μ is the overall experimental mean, and e is the experimental error consisting of the pooled interactions of both sources and families by replications. Regions are a fixed effect, and source locations and families are random effects. Differences among regions, locations within regions, and families within locations were tested for statistical significance using PROC GLM. Regional differences were tested using "locations-within-regions" as the error term, location differences were tested using "families-within-locations" as the error term, and family differences were tested against the experimental error term.

Fig. 2. Geographical variation in fall cold damage to the stem for Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*). Contour intervals represent a 30% level of risk of maladaptation from source movement. The overall mean value is shown as the contour interval between light blue and dark green.

Variance components for locations-within-regions and families-within-locations were obtained using PROC MIXED. The proportion of variation explained by regional differences in the model (R^2) was determined as the ratio of the sums of squares of regions to the total sums of squares of regions and locations-within-regions.

Within each region, variance among families was determined using the model:

$$[4] \quad Y_{ij} = \mu + B_i + F_j + e_{ij}$$

where Y_{ij} is the plot mean performance of the jth family (F) in the ith replication (B), μ is the overall experimental mean, and e is the experimental error consisting of family by replication interaction. Families were treated as random effects. Differences among families were tested for statistical significance using PROC GLM, and variance components were obtained using PROC MIXED. Family variances were not partitioned within and among locations, since many regions had too few locations with paired family samples. Differences among regions in family variances were explored by comparing family intraclass correlations, where family intraclass correlations were calculated as the ratio of the family component of variance to the total variance.

Mapping procedures

Patterns of variation in cold hardiness were mapped using procedures outlined in St. Clair et al. (2005). Briefly, a regression model was developed in which cold hardiness was a function of the environments of seed source locations. Environments were characterized using geographical, topographical, and climatic data. Geographical and topographical data were obtained from geographic information system (GIS) coverages using a 90 m digital elevation model (DEM). Climatic data were obtained from GIS coverages generated from the climate model PRISM (Daly et al. 1994). GIS was used to generate a value for cold hardiness in each grid cell using the derived regression equations and grid algebra functions in ARC/INFO. A contour interval was selected for the maps of genetic variation that corresponds with a level of risk of maladaptation of 30%. Risk of maladaptation is defined as the nonoverlap between the frequency distributions for additive genetic variances of the populations of seedlings at two different locations (Campbell 1986). This approach assumes that the native population is optimally adapted to the local environment, which may not always be the case. Nevertheless, risk of maladaptation is a valuable metric of population differentiation that takes into account mean differences as well as within-population variation. A risk value of 30% is assumed to be an acceptable level of risk for a single trait (Sorensen 1992).

Results

Considerable genetic variation in fall cold hardiness exists across the landscape for coastal Douglas-fir in western Oregon and Washington, and much of that variation is spatially structured (Tables 1 and 2; Fig. 2). Families differed significantly both among seed source locations (populations) and within locations. Population variation was large for fall cold hardiness traits. F values for locations, percentages of location variance, and Q_{st} values were much larger for fall cold damage than for growth and phenology (bud burst, bud set) traits (Table 1). Bud burst had relatively little variation among locations, but family-within-location variation was large. Population variation in total plant biomass after 2 years was low, while bud set showed an intermediate level of population differentiation.

Fall cold damage was highly correlated among tissues (Table 3). Stem damage had the highest family mean correlations with the other two tissues ($r = 0.91$ with bud damage and $r = 0.89$ with needle damage); the correlation between needle and bud damage was lower ($r = 0.81$). Family mean correlations were higher when considered across the entire study area compared with correlations within each region; for example, the correlation between stem and bud damage was 0.91 compared with an average within-region correlation of 0.72. Lower correlations within regions are partly a result of lower variation among families within regions relative to across the entire study area. Although some regions, such as the eastside Oregon Cascades, appeared to have lower correlations between tissues, no apparent geographic patterns were found between areas of strong compared with moderate correlations. Because damage to the stem can have greater consequences for whole plant growth and survival, and because correlations were strong between stem damage and the other two tissues, I will focus primarily on results for cold damage to the stem. Patterns of variation for the other tissues were nearly identical.

Populations varied clinally in fall cold damage (Fig. 2). The model for cold damage to the stem as function of seed source environments was ($R^2 = 0.63$):

$$[5] \quad \text{STDAM} = -462.9 - 5.32\text{LON} - 2.87\text{LAT} - 0.285\text{SPRFRST} - 0.00378\text{ELEV}$$

where STDAM is the percentage of fall cold damage to the stem, LON is longitude in decimal degrees, LAT is latitude in decimal degrees, SPRFRST is days since 1 January of last spring frost, and ELEV is elevation in metres. Cold damage was most strongly associated with gradients in cold-season temperatures (Table 4). As might be expected, families from colder climates suffered less freezing damage in the fall when subjected to the same temperatures in artificial freeze tests. Cold damage also varied latitudinally ($r = -0.49$), which is particularly evident along the coast. Families that suffered the greatest cold damage came from the southern Oregon Coast. Coast Range families suffered more cold damage than Cascade families of the same range of elevations and latitudes. These regional differences may be seen in the map of geographic variation (Fig. 2) as well as when comparing regional means (Table 2).

Regional differences explained a large proportion of the variation among populations in fall cold damage (R^2 =

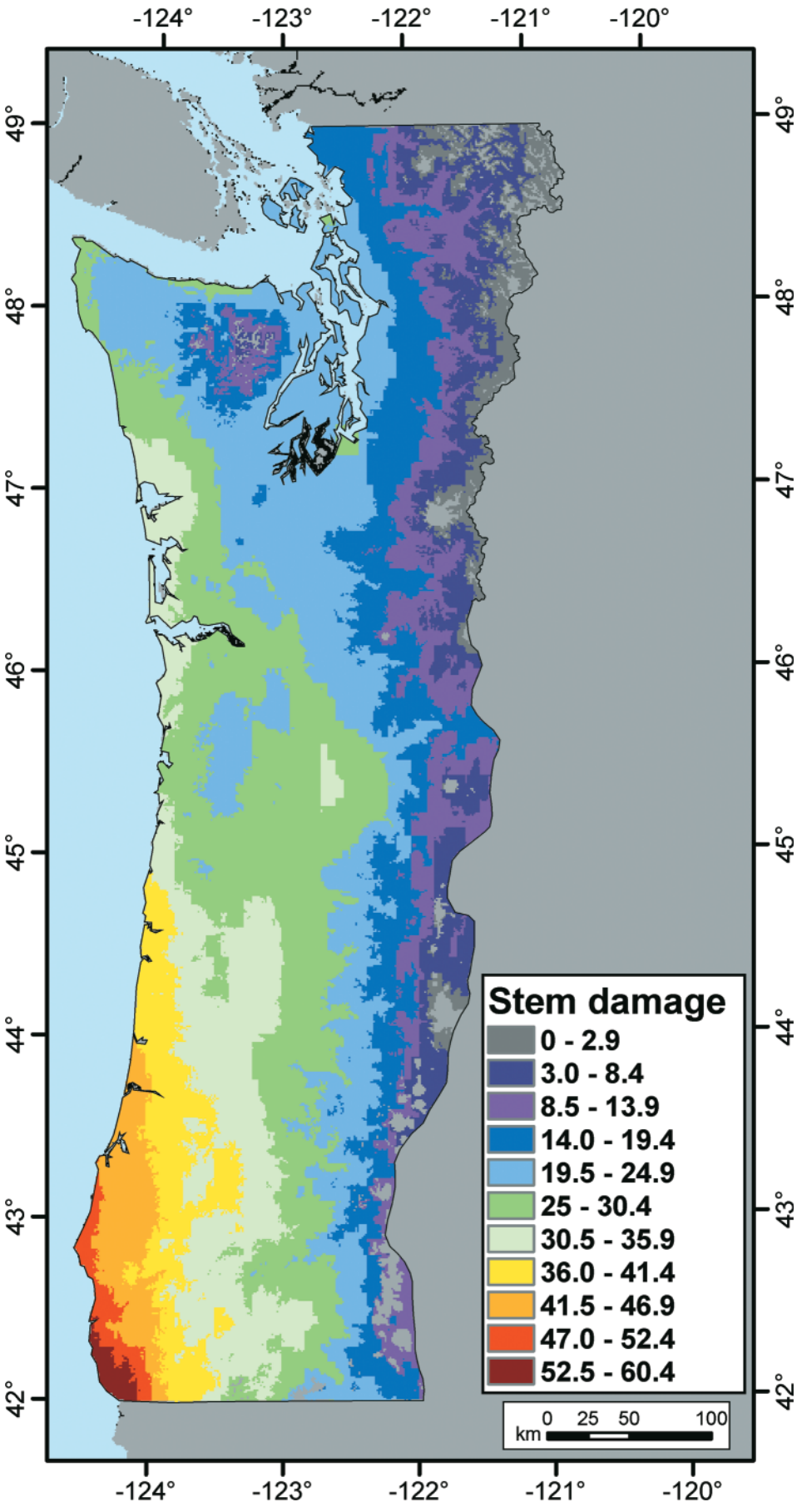


Table 3. Correlations of Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*) family means between traits across western Oregon and Washington and within each region.

Region and elevation	Stem damage with needle damage	Stem damage with bud damage	Needle damage with bud damage	Stem damage with bud set	Stem damage with bud burst	Stem damage with biomass
Correlations of family means overall	0.89*	0.91*	0.81*	0.57*	–0.19*	0.16*
Correlations of family means within						
Oregon Klamath Mountains low	0.84*	0.92*	0.84*	0.25	0.26	–0.25
Oregon Klamath Mountains high	0.81*	0.86*	0.73*	0.56*	–0.29*	0.47*
Oregon Coast south low	0.82*	0.80*	0.64*	0.61*	0.28*	–0.25*
Oregon Coast south high	0.73*	0.94*	0.71*	0.57*	0.23	–0.42
Oregon Coast north low	0.84*	0.83*	0.74*	0.46*	0.00	–0.13
Oregon Coast north high	0.89*	0.86*	0.68*	0.45	–0.23	0.08
Oregon Coast eastside low (Willamette Valley)	0.72*	0.70*	0.49*	0.04	0.45*	–0.26
Washington Coast south low	0.73*	0.66*	0.33*	–0.05	–0.34*	–0.02
Washington Coast north low (Olympics)	0.78*	0.72*	0.39	–0.03	–0.03	–0.16
Oregon Cascades south low	0.52*	0.64*	0.41*	0.27	–0.20	–0.45*
Oregon Cascades south high	0.76*	0.62*	0.54*	0.46*	–0.08	0.40*
Oregon Cascades north low	0.71*	0.59*	0.40*	0.29*	–0.03	–0.06
Oregon Cascades north high	0.84*	0.66*	0.61*	0.50*	0.32*	0.46*
Oregon Cascades eastside high	0.52*	0.64*	0.33	0.24	–0.42*	0.15
Washington Cascades south low	0.73*	0.49*	0.24	0.53*	0.38*	0.17
Washington Cascades south high	0.83*	0.67*	0.54*	0.32*	0.56*	0.24
Washington Cascades north low	0.81*	0.70*	0.59*	0.34*	0.28	0.15
Washington Cascades north high	0.78*	0.72*	0.70*	0.27	–0.16	0.18
Average within-region correlation	0.76	0.72	0.55	0.34	0.05	0.05

Note: *, family mean correlation is significantly different from zero at $p \leq 0.05$.

0.59–0.62; Table 5). Regional differences explained less variation in growth and phenology traits. As expected, population variation in all traits was lower within regions relative to the entire study area (e.g., variation among locations-within-regions and Q_{st} values were smaller). As before, families-within-locations differed significantly for all traits.

Although strong correlations were found between fall cold damage and cold-season temperatures across the study area, correlations were weaker and less consistent within regions (Table 4). For example, the average within-region family mean correlation between stem damage and winter minimum temperature was 0.28 compared with 0.67 for the family mean correlation across all of western Oregon and Washington.

Regions differed in the amount of family variation in fall cold damage (Table 6). For stem damage, family intraclass correlations ranged from 0.07 to 0.40. In some cases, particularly for bud damage, families differences were not significant at $p = 0.05$ (i.e., the family intraclass correlation was not significantly different from zero). Family variation in cold damage was low at lower elevations of the Oregon and southern Washington Cascades. Family variation was lower in the Oregon Cascades relative to the Oregon Coast Range. Other regions with low family variation in cold damage included the Washington Coast Range, the eastside of the north Oregon Coast Range (Willamette Valley), and the eastside of the Oregon Cascades.

Fall cold damage was moderately correlated with later bud set (Table 3; $r = 0.57$). Populations from colder climates generally set bud earlier and suffered less fall cold damage. The correlations between cold damage and bud burst or biomass were weak ($r = –0.19$ and $r = 0.16$, respectively). Within regions, family mean correlations were variable; the correlation between stem damage and bud set ranged from –0.05 to 0.61, the correlation between stem damage and bud burst ranged from –0.42 to 0.56, and the correlation between stem damage and biomass ranged from –0.45 to 0.47. Trade-offs between growth and fall cold damage (i.e., positive correlations) are most evident at the higher elevation regions of the Klamath Mountains and Oregon Cascades.

Discussion

Substantial clinal genetic variation in fall cold hardiness was found across the range of Douglas-fir in western Oregon and Washington. Clines varied with both latitude and longitude, and were most strongly associated with cold-season temperatures. Coast Range populations suffered greater cold damage than Cascade populations of the same approximate elevation and latitudes. These patterns of variation match those of earlier studies involving smaller geographic ranges and fewer populations. When grown together in a common garden, families from breeding zones near the Oregon Coast suffered greater fall cold damage from natural frost events or in artificial freeze tests than families from breeding zones to the east in the Cascades (Loopstra and Adams 1989; O'Neill et al. 2001). Latitudinal variation was found in 10 populations from the Coast Range grown in a common garden and damaged by fall frost (Campbell and

Table 4. Correlations of family means for Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*) between fall cold damage to the stem and climate variables overall and within each region.

Region and elevation	Annual avg. temperature	Winter min. temperature	Summer max. temperature	Date of last spring frost	Date of first fall frost	Annual precipitation	Winter precipitation[a]	Summer precipitation[b]
Correlations of family means overall	0.65*	0.67*	0.30*	–0.52*	0.65*	0.09*	0.17*	–0.35*
Correlations of family means within								
Oregon Klamath Mountains low	0.19	0.49*	–0.24	–0.61*	0.65*	0.60*	0.62*	0.31*
Oregon Klamath Mountains high	0.54*	0.57*	0.00	–0.50*	0.51*	0.27*	0.30*	0.01
Oregon Coast south low	0.33*	0.45*	–0.41*	–0.52*	0.51*	0.25*	0.22	0.12
Oregon Coast south high	0.50	0.35	0.27	–0.25	0.25	0.12	0.09	0.24
Oregon Coast north low	0.47*	0.38*	0.19	–0.43*	0.49*	–0.19	–0.18	–0.31*
Oregon Coast north high	0.48	0.09	0.62*	–0.70*	0.61*	–0.38	–0.34	–0.51
Oregon Coast eastside how (Willamette Valley)	0.37	0.34	0.35	–0.03	0.18	–0.09	–0.14	–0.01
Washington Coast south low	0.17	–0.06	0.28	0.16	–0.19	–0.44*	–0.44*	–0.39*
Washington Coast north low (Olympics)	0.11	–0.23	0.36	0.05	–0.01	–0.10	–0.08	–0.14
Oregon Cascades south low	–0.05	–0.11	0.10	0.20	–0.10	0.19	0.23	0.07
Oregon Cascades south high	0.58*	0.59*	0.47*	–0.61*	0.59*	0.25*	0.28*	0.15
Oregon Cascades north low	0.12	0.23	0.04	–0.02	0.05	0.10	0.04	0.18
Oregon Cascades north high	0.58*	0.63*	0.26*	–0.55*	0.54*	0.26*	0.22	0.28*
Oregon Cascades eastside high	0.18	–0.02	0.33	–0.18	0.20	–0.22	–0.17	–0.43*
Washington Cascades south low	0.38*	0.40*	0.00	–0.40*	0.51*	–0.26	–0.33	0.14
Washington Cascades south high	0.38*	0.41*	0.29*	–0.57*	0.56*	0.30*	0.32*	0.26
Washington Cascades north low	0.39*	0.44*	0.07	–0.36*	0.32*	–0.33*	–0.36*	–0.12
Washington Cascades north high	0.04	0.05	–0.05	–0.12	0.10	–0.07	–0.12	0.31
Average within-region correlation	0.32	0.28	0.16	–0.30	0.32	0.01	0.01	0.01

Note: *, family mean correlation is significantly different from zero at $p \leq 0.05$.

[a]Total precipitation during the months of December, January, and February.

[b]Total precipitation during the months of June, July, and August.

Table 5. Results from analyses of variance for differences among regions, seed source locations-within-regions, and families-within-locations across western Oregon and Washington for Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*).

Trait	Overall mean	*F* value for regions	*F* value for locations-within-regions	*F* value for families-within-locations	R^2 for regions[a]	Total variance	Percentage of total variance			
							Location	Family (L)	Error	Within-region Q_{st}[b]
Stem cold damage (%)	24.9	58.34***	1.96***	1.47***	0.60	233.8	21	7	72	0.34
Needle cold damage (%)	45.2	55.72***	1.81***	1.93***	0.59	348.6	20	14	66	0.20
Bud cold damage (%)	48.5	62.49***	1.77***	1.41**	0.62	249.6	17	6	77	0.31
Bud burst (d)[c]	107	17.18***	1.23	3.55***	0.31	25	17	25	58	0.10
Bud set (d)[c]	274	32.48***	1.48**	2.11***	0.46	72	12	19	69	0.09
Bud set year 2 (d)[c]	225	32.91***	1.19	1.56***	0.46	333	6	9	84	0.11
Biomass (g)	12.5	10.39***	1.36*	1.93***	0.21	2.8	8	18	74	0.07

Note: *, statistically significant at $p = 0.05$–0.01; **, statistically significant at $p = 0.01$–0.001; ***, statistically significant at $p < 0.001$.

[a]Amount of variation explained in the model by the fixed effect of regions.

[b]Defined as the proportion of the total genetic variation in quantitative traits found among populations (see text).

[c]Number of days since 1 January.

Sorensen 1973); the southernmost population (Coos Bay, Oregon; 43.3°N latitude) suffered 78% damage, whereas the northernmost source (Soleduck, Washington; 48.0°N latitude) suffered 10% damage. Families from low elevations suffered more cold damage than those from high elevations within breeding zones in southern Oregon (Loopstra and Adams 1989). Elevation has also been important in explaining variation among interior Douglas-fir populations from the northern Rockies (Rehfeldt 1979, 1982, 1983).

Large population differences across the landscape corresponding with environmental gradients are evidence that natural selection has been important in determining genetic variation in fall cold hardiness, particularly when those gradients make sense for the trait in question (i.e., cold hardiness corresponding with gradients in temperature). Population differences in fall cold hardiness found in this study (as measured by *F* values, percentages of total variance, or Q_{st}) were larger than in other traits of bud set, bud burst, growth, emergence, or partitioning (Tables 1 and 5; see also Table 2 in St. Clair et al. 2005).

Q_{st} has been used to estimate the magnitude of natural selection as a force in population differentiation relative to genetic drift and migration, as inferred from neutral molecular genetic markers and quantified by F_{st} (Spitze 1993; Merilä and Crnokrak 2001; McKay and Latta 2002). If Q_{st} exceeds F_{st}, then directional selection is presumed to play a more important role in population differentiation than neutral processes. Estimates of F_{st} for coastal Douglas-fir range from 0.022 (McKay and Latta 2002, citing data from western British Columbia from Yeh and O'Malley 1980) to 0.071 (Howe et al. 2003, citing data from California to British Columbia from Li and Adams 1989). Q_{st} values for fall cold hardiness found in this study (0.53–0.67) are up to 30-fold larger than published F_{st} values for this species across a comparable range, and are 8-fold larger than neutral differentiation in Douglas-fir rangewide. Furthermore, the strength of the relationship between cold hardiness and the environment, as measured by correlations and by the amount of variation explained by regressions of traits on environmental variables, was stronger than that of all other traits examined to date except bud set (St. Clair et al. 2005). In this context, fall cold hardiness appears to be a particularly important response for the adaptation of Douglas-fir to temperature gradients.

Questions of landscape scale are relevant to discussions of population differentiation and local adaptation. With respect to adaptive traits, how local is local? Population differentiation was much less within regions than across the entire area (Table 1 compared with Table 5). Clearly, regional environmental differences are important to the adaptation of Douglas-fir for fall cold hardiness, as well as growth and phenology traits (albeit to a lesser degree). Still, considerable population variation was present within regions, particularly for cold hardiness. For growth and phenology traits, however, higher family-within-population variation compared with population variation, and Q_{st} values equal to or slightly greater than F_{st} indicate that natural selection for these traits is less important for the adaptation of Douglas-fir populations to local environments within regions. Volis et al. (2005) recently showed the importance of spatial scale in evaluating adaptive differentiation. In a study of 20 populations of wild barley, they found Q_{st} values differed from

Table 6. Family intraclass coefficients overall and within each region and elevation for Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*).

Region and elevation	Stem damage	Needle damage	Bud damage	Bud burst	Bud set	Biomass
All of western Oregon and Washington	0.55*	0.60*	0.52*	0.52*	0.49*	0.32*
Within regions						
Oregon Klamath Mountains low	0.34*	0.48*	0.34*	0.51*	0.39*	0.08
Oregon Klamath Mountains high	0.34*	0.42*	0.32*	0.31*	0.34*	0.29*
Oregon Coast South low	0.20*	0.24*	0.20*	0.30*	0.38*	0.18*
Oregon Coast South high	0.28*	0.21*	0.39*	0.00	0.28*	0.13
Oregon Coast North low	0.32*	0.37*	0.25*	0.44*	0.22*	0.19*
Oregon Coast North high	0.40*	0.49*	0.31*	0.22*	0.10	0.34*
Oregon Coast Eastside low (Willamette Valley)	0.16*	0.24*	0.16*	0.30*	0.17*	0.26*
Washington Coast South low	0.15*	0.27*	0.12*	0.51*	0.06	0.41*
Washington Coast North low (Olympics)	0.19*	0.30*	0.12*	0.38*	0.19*	0.23*
Oregon Cascades South low	0.18	0.19*	0.08	0.10	0.26*	0.39*
Oregon Cascades South hgh	0.25*	0.36*	0.10*	0.42*	0.45*	0.22*
Oregon Cascades North low	0.16*	0.28*	0.05	0.54*	0.33*	0.22*
Oregon Cascades North high	0.20*	0.42*	0.13*	0.52*	0.44*	0.39*
Oregon Cascades Eastside high	0.07	0.20*	0.00	0.56*	0.00	0.16*
Washington Cascades South low	0.19*	0.13*	0.05	0.31*	0.13*	0.13*
Washington Cascades South high	0.24*	0.33*	0.29*	0.40*	0.26*	0.35*
Washington Cascades North low	0.38*	0.41*	0.24*	0.46*	0.24*	0.43*
Washington Cascades North high	0.19*	0.33*	0.07*	0.49*	0.35*	0.25*
Average within-region	0.24	0.32	0.18	0.38	0.26	0.26

Note: *, family intraclass correlation is significantly different from zero at $p \leq 0.05$.

F_{st} values across populations in different regions, but not within regions. My study shows that the degree of local adaptive differentiation also depends on the trait. The implications for managing genetic resources are that seed movement guidelines and breeding zones might need to be more restrictive when considering variation in fall cold hardiness compared with growth and phenology traits. The regional stratification based on ecoregions and elevation appeared to be adequate for minimizing population differences within regions for growth and phenology, but perhaps not fall cold hardiness.

One caveat, however, deserves consideration when determining current and future transfers of genetic material. Natural selection is necessarily historical; that is, adaptive differentiation is a consequence of natural selection in past environments. Furthermore, adaptive differentiation may be a consequence of natural selection in rare, extreme environments. In other words, the rare cold events of the past may have shaped the population variation observed today, and today's populations may not be best adapted to current or future environments. This may be particularly true given predicted climate change. Genetic resource managers may be willing to accept a higher level of risk of damage or loss from future rare, extreme cold events than may be indicated by current population structure, and, given climate change, those events may become less common.

Although natural selection has lead to population differentiation in fall cold hardiness, considerable genetic variation also exists within populations (Tables 1 and 5). This may reflect high levels of gene flow from wind pollination, or spatial or temporal heterogeneity of natural selection. Family variation within populations provides the raw material for future natural selection. Considerable genetic variation also exists within regions (Table 6). This variation is available for improving cold hardiness in managed populations through selection and breeding in tree improvement programs. I found significant family differences in fall cold damage in nearly all regions, with family intraclass correlations similar to other traits; unfortunately, the design of this study was not conducive to estimating heritabilities (because of sampling different families in different years and problems with adjusting for year effects on individual trees). Previous studies of fall cold hardiness in coastal Douglas-fir found individual heritabilities ranging from zero (nonsignificant family differences) to high (0.76), but mostly moderate and comparable to heritabilities for commonly selected growth traits (Aitken et al. 1996; Aitken and Adams 1997; O'Neill et al. 2000, 2001). Thus, the potential exists to breed for increased fall cold hardiness. Screening families for cold hardiness can be done in seedling tests (Anekonda et al. 2000), and cold-susceptible families can be eliminated before field testing. O'Neill et al. (2000) found strong genetic correlations between fall frost damage at seedling and sapling stages. Alternatively, cold-susceptible families may be maintained in breeding populations and seed orchards, but not deployed on frost-prone sites.

Several studies have indicated that heritabilities are higher in breeding zones in the Coast Range than in the Cascades (Aitken et al. 1996; Aitken and Adams 1996; O'Neill et al. 2000, 2001). My results support these observations, but only if comparing regions of similar elevations. Family intraclass-correlation coefficients were higher in the Coast Range of Oregon and southwestern Washington compared with Cascade regions of similar elevations (average of 0.26 compared with 0.17). But family intraclass correlations were greater at high compared with low elevations within regions

(average of 0.29 compared with 0.19). O'Neill et al. (2000) hypothesized that lower heritabilities in the Cascades may be a consequence of harsher environments leading to greater stabilizing selection for cold hardiness, which results in reduced genetic variation. Extending this rationale, higher elevation regions should be most severe with respect to selection pressures, and, thus, less genetic variation should be evident; this did not appear to be the case in my study. An alternative hypothesis is that spatial and temporal heterogeneity for cold is greater in those regions with higher levels of family variation in cold damage, including the Oregon and southern Washington Coast Range compared with the Cascades, high compared with low elevations, and the Klamath Mountains and North Cascades (both high and low elevations). For example, the transition from maritime to more continental climates may be sharp in the Coast Range given combined influences of the fog belt and rain shadows. Another hypothesis to explain higher levels of family variation at higher elevations in the Oregon Coast Range and Cascades is gene flow from low to high elevations because of prevalent westerly winds.

Tradeoffs between growth and cold hardiness are often found at the population level at larger geographic scales (Howe et al. 2003). The family mean correlation between fall cold damage and biomass across the range of Douglas-fir in this study, however, was weak ($r = 0.16$). Positive genetic correlations between cold damage and growth within regions or breeding zones may make it difficult to simultaneously breed for large trees and increased cold hardiness. Family mean correlations in this study were inconsistent among regions, although there was some indication of unfavorable correlations between growth and cold damage in higher elevation regions of the Klamath Mountains and Oregon Cascades ($r \geq 0.40$). Perhaps tradeoffs are more likely within regions of more variable or severe climates. Aitken et al. (1996) found no consistent relationship between fall cold damage and height growth in saplings in a Washington coastal breeding zone, but a weak unfavorable correlation between fall cold damage and height in a Washington Cascades breeding zone (average $r_A = 0.38$).

Spring cold hardiness was not directly measured in this study. Bud burst was used as a surrogate for spring cold hardiness based on the previously reported high correlation between them (Aitken and Adams 1997; O'Neill et al. 2000). As discussed, population variation in bud burst, and indirectly spring cold hardiness, was low, and the correlation with environment was weak (St. Clair et al. 2005). Bud burst was most strongly correlated with summer precipitation and maximum summer temperatures; in this respect it appears to be an adaptation for early growth prior to the onset of seasonal summer drought (St. Clair et al. 2005). The correlation between bud burst and spring temperatures or frost dates is weak. Much of the variation in bud burst is within populations, suggesting that natural selection pressures for spring cold hardiness have been weaker than for fall cold hardiness, even in the presence of identical rates of gene flow.

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